10-Hydroxy-cis- and 10-Hydroxy-trans-paspalic Acid Amide: New Alkaloids from Claviceps paspali

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J. Nat. Prod., **1993**, 56 (6), 810-814• DOI:
10.1021/np50096a002 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 6, pp. 810-814, June 1993

10-HYDROXY-*CIS*- AND 10-HYDROXY-*TRANS*-PASPALIC ACID AMIDE: NEW ALKALOIDS FROM *CLAVICEPS PASPALI*

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ABSTRACT.—10-Hydroxypaspalic acid amide [**1**] and 10-hydroxy-*cis*-paspalic acid amide [**2**] were found as metabolites of the postproduction phase of submerged fermentation of *Claviceps paspali*. Structure revision is based on comparison with ^{1}H- and ^{13}C-nmr data of *cis*-paspalic acid and agroclavine I.

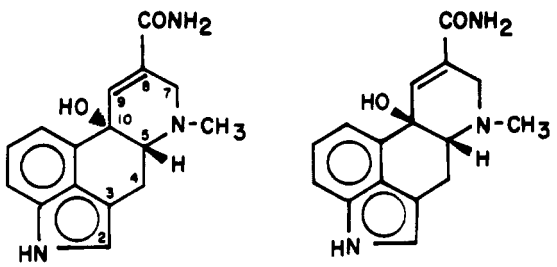
Two of our latest papers (1,2) are concerned with the submerged fermentation of *Claviceps paspali* Stevens et Hall (Clavicipitaceae) strain MG-6. The main products of this fungus are lysergic acid-α-hydroxyethylamide and ergine, the starting compounds for preparation of free lysergic acid and other derivatives. It was found that these alkaloids are formed mainly in the production and degradation phases of submerged fermentation. In the postproduction phase they undergo two biooxidative reactions that yield mostly 8-hydroxyderivatives of ergine and erginine. 10-Hydroxypaspalic acid amide [**1**] was found to be another product of this phase, and some data characterizing its structure were published earlier (1).

Furthermore, the isolation of compound **1** made it possible to complete the current biosynthetic scheme for the production of simple lysergic acid derivatives. It is assumed that the 10-hydroxyderivative **1** arose in a similar sequence of reactions as 8-hydroxyergine (1). This assumption is supported by the fact that 10-hydroxyagroclavine and 10-hydroxyelymoclavine were identified as unstable intermediates of agroclavine and elymoclavine oxidation to their 8-hydroxyderivatives (3,4).

Nevertheless, there are still about five alkaloids or their degradation products in the fermentation broth which remain unknown. The aim of this work was to determine the structure of the last of the four main alkaloids of the postproduction phase. Isolation of an isomer of **1** was the reason for the stereochemical reinvestigation.

RESULTS AND DISCUSSION

It has been mentioned above that the fermentation medium of *C. paspali* contains, besides about ten minor components, four main products synthesized in the postproduction phase. Formation of both **1** and **2** during submerged cultivation (Figure 1) gives the same profile as that of the formation of 8-hydroxyergine published earlier (2). The chromatogram of 31-day-old culture broth (Figure 2) documents the composition of the alkaloid



1 **2**

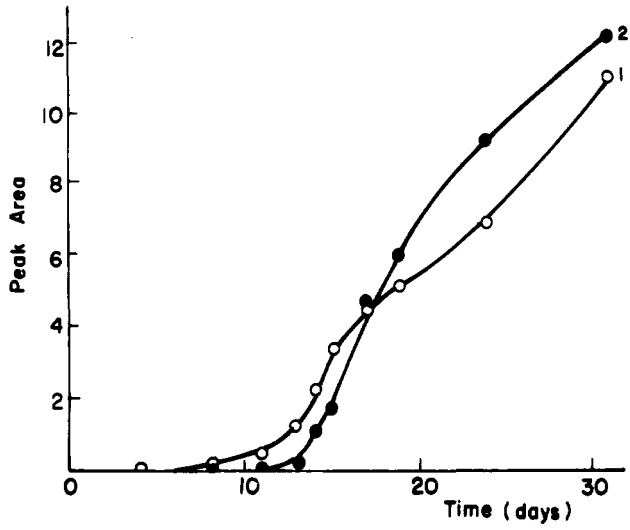


FIGURE 1. Formation of **1** (○) and **2** (●) during submerged fermentation of *Claviceps paspali* MG-6 (determined by hplc).

mixture. In addition to 8-hydroxyergine, 8-hydroxyerginine, and 10-hydroxypaspalic acid amide **1**, there is another compound **2** with much lower retention time on reversed-phase chromatography.

On the basis of their eims (Table 1), compounds **1** and **2** were recognized as isomers

TABLE 1. Eims of Compounds **1** and **2** and 8-Hydroxyergine.

m/z	Relative Intensity		
	Compound		
	1	**2**	8-Hydroxyergine
283	42	23	65
267	100	90	—
266	47	—	—
265	43	—	6
264	29	—	14
248	28	—	18
240	8	5	94
237	—	—	26
235	33	25	—
223	46	48	17
221	77	91	24
211	32	29	—
207	65	100	8
205	31	40	8
199	31	28	—
196	40	54	30
180	47	72	9
171	26	29	—
170	56	67	—
167	39	48	86
158	50	24	—
154	56	74	100
130	42	28	—

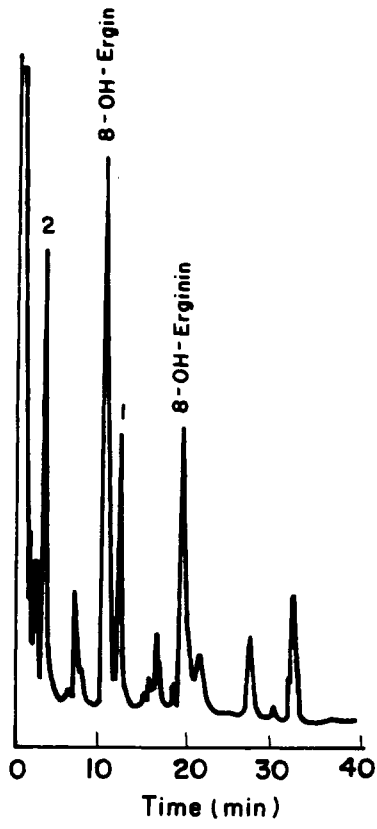


FIGURE 2. Chromatogram of alkaloid mixture (31-day-old fermentation broth of *Claviceps paspali* MG-6). Chromatographic conditions: column CGC 3×150 Tessek Separon SGX C18 5 μm; eluents (A) MeOH-H_2O-NH_3 (90:10:0.036), (B) MeOH-H_2O-NH_3 (20:80:0.036); flow rate 0.8 ml/min; detection uv at 310 nm; gradient 4% A to 54% A in 20 min, 54% A-10 min; 4% A-10 min (equil.) injection 10 μl of fermentation broth containing 0.8 mg/ml of alkaloid mixture.

of 8-hydroxyergine and 8-hydroxyerginine, respectively. Facile elimination of H_2O from the molecular ion and abundant pair peaks at *m/z* 170 and 171 support the conclusion that a hydroxyl group is located on carbon C-10 (5).

They also exhibit the same distribution of carbon signals in the ^{13}C-nmr spectra as 8-hydroxyergines, namely one N-Me, two CH_2, one CH, one quaternary C-OH, five sp^2-hybridized methines, five sp^2-quaternary carbons, and one C=O. The lower chemical shift of the carbonyl carbon in **1** and **2** (Table 1) with respect to that of 8-hydroxyergine and 8-hydroxyerginine [179.59 and 179.65 ppm (2)] is characteristic for the conjugation to a double bond. That requires a presence of a C-8 to C-9 double bond in the molecule. The assumption made on the basis of eims supports this conclusion. Therefore, **1** and **2** are $\Delta^{8,9}$-ergines carrying a 10-hydroxysubstituent and differing in the C/D ring junction only. Because of the absence of a proton at C-10, ^1H-nmr data are of no use in this case. On the other hand, the comparison of corresponding cis-trans pairs in dihydrolysergic acid methyl esters (6) and amides (7) reveals a clear trend: C-4, C-5, and C-7 resonate upfield in the cis series. The same tendency was observed in the pair agroclavine-agroclavine I (8,9) which is more similar to our compounds (Table 2). Furthermore, H-9 resonates downfield in agroclavine I (cis). Moreover, agroclavine exhibits a cross-peak between H-9 and H-12 in the NOESY spectrum, whereas

TABLE 2. ^{13}C-nmr Chemical Shifts (100 MHz, CD_3OD).

Carbon	Compound				
	1	**2**	*cis*-Paspalic acid	Agroclavine[a]	Agroclavine I[a]
C-2	120.48	120.45	120.18	117.87	117.96
C-3	111.40	109.56	109.91	112.11	111.12
C-4	22.83	18.65	18.50	26.69	16.12
C-5	68.88	67.32	60.21	63.85	58.53
C-7	57.28	53.10	51.88	60.62	53.95
C-8	136.31	136.13	135.92	133.51	130.14
C-9	134.02	138.05	136.44	119.40	123.69
C-10	69.18	71.44	40.35	40.97	39.08
C-11	132.14	132.46	132.20	132.25	133.91
C-12	114.63	115.84	116.75	112.59	115.87
C-13	123.34	124.01	124.19	122.82	123.23
C-14	112.30	111.56	110.34	108.52	108.44
C-15	134.34	134.05	132.54	132.40	133.76
C-16	127.93	127.22	127.85	126.32	126.34
C-17	171.85	171.11	173.81	20.85	20.39
N-Me	41.84	42.54	42.42	40.87	42.44

[a]In $CDCl_3$.

agroclavine I does not. Observed carbon chemical shifts of **1** and **2** (Table 2) show that C-4, C-5, and C-7 resonate upfield with **2**, and C-9 downfield. That suggests a cis C/D junction for **2**. Comparison of the ^{13}C-nmr spectra of **1** and **2** with that of *cis*-paspalic acid (paspalic acid was not available) yields a better agreement of C-4 and C-7 chemical shifts for **2** than for **1**. Noteworthy features of the ^{13}C-nmr spectrum of **2** are weak and broad signals of C-4 and C-7, hampering the determination of their multiplicity by APT and the observation of the corresponding cross-peaks in HETCOR. Nevertheless, the multiplicity of these signals was determined from the proton-coupled ^{13}C-nmr spectra obtained by gated decoupling when shorter decoupling time was used (10). Similar but smaller broadening of the same signals was observed in ^{13}C-nmr spectra of agroclavine I and *cis*-paspalic acid, for which the C/D ring stereochemistry follows unambiguously from the magnitude of $J_{5,10}$. This suggests slow conformational changes in both C and D rings at room temperature that might be diagnostic for the cis series. This effect is largest with **2**, in which both ^{1}H and ^{13}C signals are broadened [for a recent case describing broadening of ^{13}C signals only, see Murata *et al.* (11)]. That further supports our previous deduction.

Therefore, the structure of 10-hydroxy-*cis*-paspalic acid amide was assigned to compound **2**. However, this compound is identical to that reported earlier (1) and tentatively assigned the structure of 10-hydroxypaspalic acid amide. The new compound described in this paper is therefore 10-hydroxy-*trans*-paspalic acid amide [**1**].

EXPERIMENTAL

STRAIN.—The strain *C. paspali* MG-6 was isolated from the grass *Paspalum dilatatum* by Prof. H. Rochelmayer, Institute of Pharmacy, University of Mainz, Germany. It is deposited in the Collection of Microorganisms of the Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic. Media, cultivation conditions, and determination of total alkaloid and dry wt were described elsewhere (1,12).

ALKALOID SEPARATION.—Alkaloids were separated from the 31-day-old culture medium by extraction with EtOH after adjusting the pH to 8.5 by NH_4OH. A crude alkaloid mixture (9.6 g) was chromatographed on a silica column, using a gradient of MeOH (0–20%) in CH_2Cl_2, and gave 0.6 g of **1** and 0.9 g of **2**.

CHROMATOGRAPHY.—Pre-purified alkaloids were loaded on a Separon SGX C-18 column (Tessek, Czech Republic), particle size 7 μm, 25×0.8 cm i.d., and re-chromatographed under conditions described previously (2); compounds **1** (15 mg) and **2** (25 mg) were obtained. A Separon SGX C-18 column (15×0.3 cm i.d., particle size 5 μm, Tessek) was used for checking purity under the same conditions (2); capacity factors were 10.08 for **1** and 3.06 for **2**.

SPECTRAL PROCEDURES.—The positive ion eims were taken on a double sector Finnigan MAT 90 instrument by the direct inlet ei technique: ion source temperature 250°, electron energy 70 eV, ion current 1.0 mA, accelerating voltage 5.0 kV. The eims are summarized in Table 1. ^{1}H- and ^{13}C-nmr spectra were measured on a Varian VXR-400 spectrometer (400 and 100 MHz, respectively) in CD_3OD at 25°, unless otherwise stated. Digital resolution was better than 0.1 and 0.65 Hz/point for ^{1}H- and ^{13}C-nmr spectra, respectively. Chemical shifts are given in the δ scale. Carbon signal multiplicities were determined by APT or proton-coupled spectra. Standard manufacturer's software was used for 2D nmr experiments [COSY, long-range COSY (0.2 sec delay), NOESY, and HETCOR] performed on all reported compounds.

^{1}H-nmr chemical shifts (400 MHz) were obtained in CD_3OD (TMS, 25°).

Compound **1**.—^{1}H nmr δ 2.551 (s, 3H, N-Me), 2.579 (dd, $J_{4eq,5}$=4.5, $J_{4ax,5}$=11.9, 1H, H-5), 2.984 (ddd, $J_{2,4ax}$=1.7, $J_{4ax,4eq}$=14.1, 1H, H-4ax), 2.999 (dd, $J_{7ax,7eq}$=17.0, $J_{7eq,9}$=2.4, 1H, H-7eq), 3.257 (ddd, $J_{2,4eq}$=0.4, 1H, H-4eq), 3.755 (dd, $J_{7ax,9}$=1.3, 1H, H-7ax), 7.025 (dd, 1H, H-2), 7.093 (dd, $J_{12,13}$=7.2, $J_{13,14}$=8.1, 1H, H-13), 7.279 (dd, $J_{12,14}$=0.7, 1H, H-12), 7.303 (dd, 1H, H-14), 7.512 (dd, 1H, H-9).

Compound **2**.—^{1}H nmr δ 2.516 (s, 3H, N-Me), 3.058 (mt, 1H, H-4ax), 3.163 (dd, $J_{4eq,5}$=4.6, $J_{4ax,5}$=10.1, 1H, H-5), 3.221 (ddd, $J_{2,4eq}$=1.1, $J_{4ax,4eq}$=15.7, 1H, H-4eq), 3.396 (b mt, $J_{7ax,7eq}$=17.1, 1H, H-7eq), 3.460 (dmt, 1H, H-7a), 6.985 (d, 1H, H-2), 7.020 (bs, 1H, H-9), 7.172 (dd, $J_{12,13}$=7.2, $J_{13,14}$=7.9, 1H, H-13), 7.225 (dd, $J_{12,14}$=1.0, 1H, H-12), 7.261 (dd, 1H, H-14).

cis-*Paspalic acid*.—^{1}H nmr δ 2.683 (s, 3H, N-Me), 2.898 (dddd, $J_{2,4ax}$=0.7, $J_{4ax,4eq}$=15.0, $J_{4ax,5}$=10.0, $J_{4ax,10}$=0.5, 1H, H-4ax), 3.082 (ddd, $J_{2,4eq}$=0.5, $J_{4eq,5}$=4.7, 1H, H-4eq), 3.482 (ddd, $J_{5,10}$=4.8, 1H, H-5), 3.532 (ddd, $J_{7ax,7eq}$=17.5, $J_{7eq,9}$=2.2, $J_{7eq,10}$=3.8, 1H, H-7eq), 3.644 (ddd, $J_{7ax,9}$=2.0, $J_{7ax,10}$=2.8, 1H, H-7ax), 4.105 (mt, $J_{9,10}$=1.0, $J_{10,12}$=0.85, $J_{10,14}$=0.85, 1H, H-10), 6.864 (mt, 1H, H-9), 6.923 (dd, 1H, H-2), 6.938 (dd, $J_{12,13}$=7.0, $J_{12,14}$=0.85, 1H, H-12), 7.114 (dd, $J_{13,14}$=8.2, 1H, H-13), 7.178 (dd, 1H, H-14).

Agroclavine.—^{1}H nmr ($CDCl_3$) δ 1.774 (dddd, $J_{7eq,17}$=1.1, $J_{7ax,17}$=1.2, $J_{9,17}$=1.5, $J_{10,17}$=2.0, 3H, H-17), 2.497 (s, 3H, N-Me), 2.526 (ddd, $J_{4eq,5}$=4.0, $J_{4ax,5}$=11.7, $J_{5,10}$=9.4, 1H, H-5), 2.788 (ddd, $J_{2,4ax}$= 1.8, $J_{4ax,4eq}$=14.4, 1H, H-4ax), 2.923 (dddq, $J_{7ax,7eq}$=16.2, $J_{7eq,9}$=2.3, $J_{7eq,10}$=4.0, 1H, H-7eq), 3.247 (dddq, $J_{7ax,9}$=2.4, $J_{7ax,10}$=1.2, 1H, H-7ax), 3.315 (dddd, $J_{2,4ax}$=0.6, $J_{4ax,10}$=0.6, 1H, H-4eq), 3.759 (mt, 1H, H-10), 6.182 (m, 1H, H-9), 6.182 (ddd, $J_{2,NH}$=1.9, 1H, H-2), 6.987 (ddd, $J_{10,12}$=1.5, $J_{12,13}$=6.5, $J_{12,14}$=1.3, 1H, H-12), 7.112 (ddd, $J_{10,14}$=0.9, $J_{13,14}$=8.2, 1H, H-14), 7.147 (dd, 1H, H-13), 8.446 (d, 1H, NH).

Agroclavine I.—^{1}H nmr ($CDCl_3$) δ 1.637 (ddt, $J_{7ax,17}$=0.9, $J_{7eq,17}$=0.9, $J_{9,17}$=1.5, $J_{10,17}$=2.4, 3H, H-17), 2.577 (s, 3H, N-Me), 2.834 (ddd, $J_{2,4ax}$=1.6, $J_{4ax,5}$=10.6, $J_{4ax,4eq}$=14.7, 1H, H-4ax), 2.971 (ddd, $J_{2,4eq}$=0.7, $J_{4eq,5}$=4.7, 1H, H-4eq), 3.082 (ddq, $J_{7ax,7eq}$=16.8, $J_{7eq,9}$=2.1, 1H, H-7eq), 3.109 (ddq, $J_{7ax,9}$=1.1, 1H, H-7ax), 3.378 (ddd, $J_{5,10}$=4.8, 1H, H-5), 3.963 (mt, $J_{9,10}$=2.5, $J_{10,12}$=0.8, 1H, H-10), 5.528 (mt, 1H, H-9), 6.831 (ddd, $J_{2,NH}$=1.3, 1H, H-2); 6.916 (mt, $J_{12,14}$=2.4, 1H, H-12), 7.155 (mt, 2H, H-13, H-14), 8.207 (d, 1H, NH). There is a good agreement between our results and earlier published data (9).

LITERATURE CITED

1. R. Bumbová-Linhartová, M. Flieger, P. Sedmera, and J. Zima, *Appl. Microbiol. Biotechnol.*, **34**, 703 (1991).
2. M. Flieger, R. Linhartová, P. Sedmera, J. Zima, P. Sajdl, J. Stuchlík, and L. Cvak, *J. Nat. Prod.*, **52**, 1003 (1989).
3. E.H. Taylor and H.R. Shough, *Lloydia*, **30**, 197 (1967).
4. W.-N. Chan Lin, E. Ramstad, and E.H. Taylor, *Lloydia*, **30**, 202 (1967).
5. W.-N. Chan Lin, "Enzymatic Conversion of Clavine Alkaloids," Ph.D. Thesis, Purdue University, Lafayette, IN, 1967, p. 134.
6. L. Zetta and G. Gatti, *Tetrahedron*, **31**, 1403 (1975).
7. L. Zetta and G. Gatti, *Org. Magn. Reson.*, **9**, 218 (1977).
8. A.G. Kozlovsky, T.F. Solovyeva, V.G. Sakharovsky, and V.M. Adanin, *Prikl. Biokhim. Mikrobiol.*, **18**, 535 (1982).
9. V.G. Sakharovsky and A.G. Kozlovsky, *Tetrahedron Lett.*, **25**, 109 (1984).
10. P.B. Barker, A.J. Shaka, and R. Freeman, *J. Magn. Reson.*, **65**, 535 (1985).
11. M. Murata, A.-M. Legrand, P.J. Scheuer, and T. Yasumoto, *Tetrahedron Lett.*, **33**, 525 (1992).
12. V. Rylko, R. Linhartová, P. Sajdl, and Z. Řeháček, *Folia Microbiol. (Prague)*, **33**, 425 (1988).

Received 8 June 1992